Summary and Conclusion
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* Immunomodulatory activity of ten Indian medicinal plants were investigated. The plants were selected based on their medico-ethano botanical knowledge.

The plants used for the present study were as follows:


* The extracts were prepared from leaf, root and whole plant.

* In vitro preliminary biosafety studies was done for all the 10 medicinal plants selected. The cytotoxic nature of the plants was studied on Vero cell line to rule out the toxic nature of the plant compound and also to prove that they were not toxic.

* The results revealed that these plant extracts were not cytotoxic to the Vero cell line up to a concentration of 2 mg/ml. The tested concentration ranged from 2 mg/ml to 31.25 μg/ml.
All the 10 crude medicinal plants were subjected to methanolic extraction and tested for their stimulatory and inhibitory potential of lymphocyte proliferation by $^3$H thymidine incorporation. The plant extracts were tested at different concentrations ranging from 50 to 200 µg/ml.

Among the 10 plants tested in vitro for lymphocyte proliferation four plants Alternanthera sessilis, Polygala chinensis, Tribulus terrestris and Oxalis corniculata have shown stimulation of lymphocyte proliferation.

Among the four plants that have shown stimulation of lymphocyte proliferation, the plant Alternanthera has shown the highest stimulation of lymphocyte proliferation (67%) at a concentration of 100 µg/ml.

Successive extraction was carried out for the plant Alternanthera sessilis using different solvents such as n-hexane, benzene, ethyl acetate, chloroform, acetone, ethanol and water.
Our results revealed that the aqueous chloroform and ethanol extracts of *Alternanthera sessilis* have shown a higher percentage of stimulation of lymphocyte proliferation (69%, 67%, 67.5% respectively) than the extracts of n-hexane (45%), benzene (52.8%), ethylacetate (42.3%) and acetone (55%).

The aqueous extract of *Alternanthera sessilis* was fractionated with high pressure liquid chromatography, 20 fractions were collected. Among the 20 HPLC fractions tested for stimulation of lymphocyte proliferation, the fraction No.4, fraction No.11, fraction No.15, fraction No.16, fraction No.18 and fraction No.20 showed significant T-lymphocyte stimulation (72%, 65.8%, 62.3%, 68%, 71.5%, 64% respectively) at a concentration of 10 μg/ml.

The present study have shown that among the 10 crude plants extracted with methanol were tested for lymphocyte proliferation at different concentrations such as 50 μg/ml, 100 μg/ml, 150 μg/ml and 200 μg/ml, the plant *Justicia gendarussa* have shown a maximum inhibition of lymphocyte proliferation (65.2%) while the other plants *Plumbago indica*, *Aloe vera*, *Aegle marmelos* showed 60%, 62.3% and 59% at a concentration of 100 μg/ml.
Our results revealed that among the four methanolic extract which has shown inhibitory effect on lymphocyte proliferation the plant extract Justicia gendarussa have shown a maximum inhibitory effect (65.2%) on lymphocyte proliferation hence this plant extract was subjected to successive soxhlet extraction with different solvents such as n-hexane, benzene, ethyl acetate, chloroform, acetone, ethanol and water.

Our results showed that the aqueous chloroform and ethanol have shown a higher percentage 66%, 63.5%, 64% on inhibition of lymphocyte proliferation respectively than the n-hexane (55.6%), benzene (43%), ethylacetate (45%) and acetone (56%) at a concentration of 50 µg/ml.

Thus this study revealed that the aqueous extract of Justicia gendarussa has shown a high inhibition on lymphocyte proliferation and hence it was subjected to fractionation with HPLC, 17 fractions were collected. Among the 17 fractions tested for inhibition of lymphocyte proliferation, the fraction No.6, fraction No.7, fraction No.10, fraction No.14 and fraction No.15 showed 60%, 61%, 64%, 65% and 68% respectively of inhibition of lymphocyte proliferation at a concentration of 10 µg/ml.
RT PCR was standardized to detect the *in vitro* induction of cytokines such as IFN-γ and IL-4, for all the crude medicinal plant methanolic extracts.

Our study revealed that the methanolic extracts of *Polygala chinensis*, *Alternanthera sessilis*, *Plumbago indica* and *Aloe vera* showed IFN-γ induction while the other six extracts *Tribulus terrestris*, *Oxalis corniculata*, *Evolvulus alsinoides*, *Aegle marmelos*, *Vitex negundo*, and *Justicia gendarussa* did not show IFN-γ induction.

Our study also revealed that among the 10 crude methanolic extract tested for IL-4 induction, *Alternanthera sessilis*, *Vitex negundo*, *Justicia gendarussa* and *Aegle marmelos* have shown IL-4 induction. While the other six plants *Tribulus terrestris*, *Evolvulus alsinoides*, *Justicia gendarussa*, *Polygala chinensis*, *Oxalis corniculata*, and *Plumbago indica* did not show IL-4 induction.

Thus in our study out of the 10 extracts tested for IFN-γ and IL-4 induction only *Alternanthera sessilis* has shown IL-4 and IFN-γ induction and hence the HPLC fractions of this extract was tested for IFN-γ and IL-4 induction.
Among the 20 HPLC fractions of *Alternanthera sessilis* tested for IFN-γ induction, fraction No.4, fraction No.11, fraction No.16, fraction No.18, fraction No.19 and fraction No.20 have shown IFN-γ induction.

Among the 20 HPLC fractions of *Alternanthera sessilis* tested for IL-4 induction the fraction No.4, fraction No.11, fraction No.16 and fraction No.18 have shown IL-4 induction.

Our study revealed that the methanolic extract of *Justicia gendarussa* did not show IFN-γ induction but it has shown IL-4 induction hence the HPLC fractions of *Justicia gendarussa* was tested for IL-4 induction among the 17 fractions tested for IL-4 induction, fraction No.4, fraction No.7 and fraction No.5 have shown IL-4 induction.

The effect of *Justicia gendarussa* on adjuvant induced arthritis rats was studied and compared with indomethacin.

Measurement of the paw volume of rats with adjuvant-induced arthritis revealed an increase in ankle diameter from days 4 which increased further up to day 19.
Increased paw diameter in the arthritic animals were significantly suppressed to near normal levels in rats treated with 600 mg/kg of *Justicia gendarussa* aqueous extract and 3 mg/kg indomethacin, the effect being almost the same as that of indomethacin. The significance between the control and experimental animals were analysed by students 't' test. It was found to be statistically significant (p<0.05).

The aqueous extract of *Justicia gendarussa* was able to diminish the inflammation occurring during the DTH response in rats in a dose dependent way. Statistically it was found to be significant (p<0.05).

The oral administration of *Justicia gendarussa* extract (400 mg/kg and 600 mg/kg) resulted in suppression of haemagglutination antibody titre. The effect at 600 mg/kg was found to be higher than that at 400 mg/kg and the difference was statistically significant p<0.05.

Among the ten plants tested for lymphocyte proliferation, IFN-γ and IL-4 induction. *Vitex negundo* has shown only induction of IL-4. *Tribulus terrestris* and *Oxalis corniculata* have shown only stimulation of lymphocyte proliferation. *Alternanthera sessilis* have shown stimulation of lymphocyte proliferation and induction of both IFN-γ and IL-4. *Polygala chinensis* has shown stimulation of
lymphocyte proliferation and IFN-γ induction. *Justicia gendarussa* and *Aegle marmelos* have shown inhibition of lymphocyte proliferation and IL-4 induction. *Plumbago indica* and *Aloe vera* have shown inhibition of lymphocyte proliferation and IFN-γ induction.

The plant *Alternanthera sessilis* has shown a maximum stimulation of lymphocyte proliferation and it has also induced the IFN-γ and IL-4 cytokines. Fraction No.15 has shown only stimulation of lymphocyte proliferation and fraction No.20 has shown stimulation of lymphocyte proliferation and induction of IFN-γ. The fraction 19 has shown only IFN-γ induction. Fractions 4, 11, 16 and 18 have shown both IFN-γ and IL-4 induction and also stimulation of lymphocyte proliferation. Since the crude plant extracts and their fractions have shown stimulation of lymphocyte proliferation and induction of cytokines such as IFN-γ and IL-4, the plant *Alternanthera sessilis* can be considered to have immunostimulant activity. The aqueous extract and fractions of *Alternanthera sessilis* have shown a maximum activity on stimulation of lymphocyte proliferation and it has induced both IFN-γ and IL-4. This suggests that the immunostimulating substances are essentially water soluble in nature and a higher concentration of active fraction was present in the aqueous fractions. The results obtained in this study indicated the *Alternanthera sessilis* has the capacity of increasing the cellular immune response. Further *in vivo* studies are to be carried out for
the plant *Alternanthera sessilis* to find its action on humoral immune response.

The plant *Justicia gendarussa* has shown a maximum inhibition of lymphocyte proliferation and it has induced IFN-γ and not IL-4 cytokines. The fraction No.6, 7, 10, 14, 15 have shown inhibition of lymphocyte proliferation and the fraction No.4, 7 and 5 have shown IL-4 induction. Since the crude plant extract of *Justicia gendarussa* and its fraction have shown inhibition of Lymphocyte proliferation and induction IL-4 cytokine, thus this plant *Justicia gendarussa* can be considered for its immunosuppressive activity. A maximum of immuno suppressive activity was present in the aqueous extract.

The aqueous extract of *Justicia gendarussa* significantly suppressed the increased paw diameter in adjuvant induced arthritic animals, the effect was almost the same as that of indomethacin. *Justicia gendarussa* was able to diminish the DTH response in a dose dependant way. The oral administration of *Justicia gendarussa* resulted in suppression of haemagglutination antibody titre in a dose dependant way. Based on the results of the study of *Justicia gendarussa* plant, we came to the conclusion that the leaf extract of *Justicia gendarussa* has potential anti inflammatory activity and a suppressive action on humoral antibody response and thus provides
a scientific basis for the utilization of this plant in traditional medicine for the treatment of inflammatory diseases. Phytochemical analysis of the active fractions and the exact mechanism of action are to be studied in future.